STUDIES ON COMPLEMENT FIXATION

III. THE EFFECT OF HEAT ON COMPLEMENT-FIXING ANTIBODIES

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This paper presents studies on the effect of heat on three types of complement-fixing substances: those present in syphilitic serum; those present in rabbits immunized with purified proteins, and those found in animals as a result of bacterial immunization.

These studies were undertaken as a result of an accidental observation made in this laboratory during the early part of the summer of 1920. A number of Wassermann positive syphilitic serums intended for inactivation were accidentally placed in a water bath of 62 C. (instead of 56 C.) and permitted to remain for 15 minutes. Instead of discarding these serums, they were examined for complement-fixing substances side by side with parts of the same serums which were inactivated for half an hour at 56 C. It was desired to corroborate the prevalent view that a temperature of 62 C. destroys the complement-fixing substance in syphilitic serum.

The Wassermann tests were made in duplicate, with a cholesterinized antigen with a half hour fixation period in the water bath and an alcoholic antigen with a 4 hour fixation period in the icebox. It was found that the tests carried out with water bath fixation were negative, indicating complete destruction of the complement-fixing substances, while those carried out with icebox fixation gave only slightly weaker results than the same serums which underwent a half hour heating at 56 C.

That the difference in the results of the two sets of tests was not due alone to the different antigens employed was soon shown by preliminary experiments. These experiments furthemore established without question that a fixation period of 4 hours in the icebox gave results altogether different from a fixation period of a half hour in the water bath when employing serums exposed to different temperatures. We thus had a problem worthy of experimental investigation. It will be recalled that Noguchi [1] has pointed out the marked destruc-

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[1] Serum Diagnosis of Syphilis, Ed. 2, p. 97.

tion of syphilitic antibodies during inactivation; the antibody content he found to be reduced to about one-fourth of the total. He used in his test a half hour fixation period in the water bath, and it seemed likely that a 4 hour period in the icebox would have given him altogether different results. More recently, Kolmer, Rule and Trist,[2] also, studied the effect of heat on complement-fixing antibodies in syphilitic serums. They found that temperatures ranging from 62 to 65 C. destroy these antibodies completely. And with regard to inactivation, they recommend a 15 minute instead of the usual 30 minute period at 56 C. This, because of the high destruction of complement-fixing substances during the latter period of inactivation. They, however, also used water bath fixation.

In view of these considerations, a series of studies on the effect of heat on complement-fixing substances was undertaken with a particular view to determining the effect of the mode of fixation on the destructibility of these antibodies.

THE EFFECT OF HEAT ON COMPLEMENT-FIXING SUBSTANCES IN SYPHILITIC SERUMS[3]

The plan of these studies was to find the relative number of complement-fixing substances in unheated serum and in the same serum heated at various temperatures in the water bath for different periods. All tests were carried out in duplication with a 1 hour fixation period in the water bath, 37.5 C., and a 4 hour period in the icebox, 8-12 C.

The complement fixation tests were carried out in the usual manner with a sheep-cell system and guinea-pig complement. The complement, amboceptor, antigen and sheep-cell suspension were used in 0.1 c c quantities while the immune serum was used in every case in the following dilutions: 0.01, 0.007, 0.004, 0.003, 0.002, 0.001, 0.0005, 0.0003, and 0.0001 c c. Two units of complement and 2 units of amboceptor were used, and the tests were uniformly read after permitting the racks to remain in the icebox over night.

The syphilitic serums in these experiments were positive Wassermann serums left over from those sent to this laboratory for examination. The antigens employed were (1) an alcoholic extract of beef heart; (2) the same antigen cholesterinized; (3) an alcoholic extract of guinea-pig hearts; and (4) a Noguichi antigen. These antigens

[2] Jour. of Syph., 1920, 4, p. 641.

[3] For preliminary report see Kahn, R. L., and Boyd, A. G.: Abstr. of Bacteriol., 1921, 5, p. 17.

are the same as the first 4 antigens employed in study II.[4] Five times the quantity of these antigens employed in the tests were neither anticomplementary nor hemolytic.

Effect of Mode of Fixation on Velocity of Thermal Destruction of Syphilitic Antibodies.—For the sake of uniformity, the velocity of thermal destruction of syphilitic antibodies was measured by subjecting the serums in practically all cases to the same temperatures and periods. The following were chosen: 5, 15 and 30 minutes at 56 C. and 10 and 20 minutes at 62 C.

At the very beginning of these experiments a phenomenon was encountered which we have not as yet been able to explain fully. We found that some serums appear to possess greater fixing powers after being heated up to 30 minutes at 56 C. than before heating; this occurred only when icebox fixation was resorted to.

An increase in fixability in inactivated serum compared with raw serum is what might be expected in such cases when the latter contains considerable amounts of native complement and hemolysin. The destruction of complement and partial destruction of hemolysin by heat compared with slight destruction of syphilitic antibodies would tend to render the tests stronger after inactivation. The fact, however, that this increase in fixability after heating, takes place only when icebox fixation is employed is difficult to explain.

Table 1 illustrates this point. The antigen was an alcoholic extract of beef hearts and the serum dilutions ranged from 0.01 to 0.0001 c c. The degree of fixation is reduced to a numerical value by adding the total number of plus signs in each case. The antibody loss or gain due to heating is computed accordingly. Of 32 serums tested, 10 showed gains in antibody content after heating up to 30 minutes at 56 C. These gains ranged from 5 to 125%. The others showed either no antibody loss at this temperature or only about 10% loss; this, only when a fixation period of 4 hours in the icebox was employed. With the employment of water-bath fixation, the average antibody loss due to heating was 32%.

The velocity of thermal destruction of syphilitic complement fixing substances was next studied, employing a cholesterinized antigen of beef hearts. Thirty-four serums were tests and the experiments conducted as outlined in table 1. The results up to 30 minutes heating at 56 C. were similar to the findings with the alcoholic antigen, except that a

[4] Kahn, R. L., and Olin, R. M., Jr.: Jour. Infect. Dis., 1921, 29, p. 630.

comparatively fewer number showed gains in antibody content. Ten serums tested with the alcoholic extract of guinea-pig hearts also gave results similar to the alcoholic extract antigen of beef hearts. Eleven serums tests with the Noguchi antigen showed considerable loss due to heating with either icebox or water-bath fixation, more so, however, with the latter.

TABLE 1

THE EFFECT OF THE MODE OF FIXATION ON THE VELOCITY OF THERMAL DESTRUCTION OF SYPHILITIC ANTIBODIES. TESTS WITH ALCOHOLIC-EXTRACT ANTIGEN OF BEEF-HEART

Time and Temperature of Heating	Mode of Fixation	Serum Cc									Total Plus Signs	Effect of Heating	
		0.01	0.007	0.004	0.003	0.002	0.001	0.0005	0.0003	0.0001		Antibody	
												Loss %	Gain %
0	Water bath*	3†	3	2	1	1	—	—	—	—	10	..	..
	Icebox.....	4	4	2	2	1	—	—	—	—	13	..	..
5 min. at 56 C.	Water bath.	3	2	1	1	1	—	—	—	—	8	20	..
	Icebox.....	4	4	3	2	2	1	—	—	—	16	..	23
15 min. at 56 C.	Water bath.	3	2	1	1	1	—	—	—	—	8	20	..
	Icebox.....	4	4	3	3	2	1	—	—	—	17	..	30
30 min. at 56 C.	Water bath.	2	2	1	1	—	—	—	—	—	6	40	..
	Icebox.....	4	4	3	3	2	2	—	—	—	18	.	38
10 min. at 62 C.	Water bath.	1	1	—	—	—	—	—	—	—	2	80	..
	Icebox.....	4	3	3	2	1	—	—	—	—	13	0	0
20 min. at 62 C.	Water bath.	1	1	—	—	—	—	—	—	—	2	80	..
	Icebox.....	3	3	2	1	1	—	—	—	—	10	23	..

* The period of water bath fixation was 1 hour; of icebox fixation, 4 hours.
† 4 = ++++; 3 = +++; 2 = ++; 1 = +; and — = negative.

A temperature of 62 C. was found to be destructive to the complement-fixing substances in practically all serums tested. When employing alcoholic and cholesterinized antigens, the average antibody destruction after 20 minutes' heating at 62 C. was 40% with the icebox fixation and about 70% with water-bath fixation. With the Noguchi antigen marked destruction was noted at this temperature with either mode of fixation.

Table 2 summarizes the differences in antibody destruction with the 2 modes of fixation after heating for 30 minutes at 56 C. and 20 minutes at 62 C.

One of the first problems that suggested itself as a result of these findings was whether the comparatively small loss in fixability of heated serum after 4 hours' fixation in the icebox compared with 1 hour in the water bath was due primarily to the longer period of fixation or

to the colder temperature of fixation. In order to throw light on this question, a series of 30 experiments (of the total) were carried out employing the following three modes of fixation: 1 hour in the icebox, 1 hour in the water bath, and 4 hours in the icebox. It was observed that the differences in the degree of fixation after 1 hour in the icebox compared with 1 hour in the water bath was not marked. When employing the Noguchi antigen, the degree of fixation was slightly stronger after 1 hour in the water bath than after the same period in the icebox. With the alcoholic and cholesterinized antigens the tendency was for slightly stronger fixation after 1 hour in the icebox.

TABLE 2

SUMMARY OF THE EFFECT OF THE MODE OF FIXATION ON THE DESTRUCTION OF SYPHILITIC ANTIBODIES BY HEAT

No. of Serums Tested	Antigen	Mode of Fixation	Average Loss of Antibodies Due to Heating	
			After 30 Min. at 56 C., %	After 20 Min. at 62 C., %
32	Alcoholic Extract of Beef Hearts	1 hour in water bath.....	31	76
		4 hours in icebox.....	22 (gain)	42
34	Cholesterinized antigen of Beef Hearts	1 hour in water bath.....	32	64
		4 hours in icebox.....	3	41
10	Alcoholic Extract of Guinea-Pig Hearts	1 hour in water bath.....	36	83
		4 hours in icebox.....	7 (gain)	38
11	Noguchi Antigen.....	1 hour in water bath.....	29	77
		4 hours in icebox.....	16	65
Totals of 87 serums.....		1 hour in water bath.....	32	75
		4 hours in icebox.....	10 (gain)	46

As a whole it would appear that the increased fixability of 4 hours in the icebox compared with 1 hour in the water bath is due largely to the longer period of fixation.

Perhaps the outstanding feature of these experiments is the marked variations in the behavior of each serum toward heat. Two syphilitic serums subjected to the same temperatures and tested with the same antigens and the same modes of fixation, will frequently show 100% variation. The general tendency, however, is as indicated in table 2.

Summary of Results With Syphilitic Serums.—The velocity of thermal destruction of syphilitic complement-fixing antibodies was investigated, and it was observed that the mode of fixation markedly affected the results obtained. When fixation was carried out for 1 hour at water bath temperature, the heating of serums for 5, 15 and 30

minutes at 56 C. and 10 and 20 minutes at 62 C. showed progressive destruction of these antibodies, corroborating the findings of Noguchi and other investigators. When, however, fixation was carried out for 4 hours at icebox temperature, the heating of serums up to 30 minutes at 56 C. showed either a small gain or slight loss in antibody content. A temperature of 62 C. resulted in almost half of antibody destruction compared with water-bath fixation. These findings apply to alcoholic and cholesterinized antigens. When employing a Noguchi antigen, antibody destruction due to heat was found to be marked with either mode of fixation, although more so with water-bath then with icebox fixation.

With regard to the inactivation of serums to be tested, for half an hour at 56 C., the results indicate that there is no advantage in reducing this period to 10 or 15 minutes when employing alcoholic and cholesterinized antigens and icebox (4 hours) fixation. With the Noguchi antigen, however, even with icebox fixation, a reduction of the inactivation period should be of advantage.

THE EFFECT OF HEAT ON COMPLEMENT-FIXING ANTIBODIES PRODUCED BY PROTEIN IMMUNIZATION [5]

Having shown to what extent the mode of fixation affected the heat destruction of complement-fixing substances of syphilitic serums, it seemed worth while to extend these studies to specific complement-fixing antibodies produced in rabbits by protein immunization.

Four rabbits were employed in this series. Two were immunized with edestin from hempseed and 2 with phaseolin from the kidney bean. The methods of immunization are fully described in the first paper of this series.[6] The complement-fixation tests were conducted in the same manner as with the syphilitic serums. The specific protein antigens were tested with the rabbit immune serums, both in a raw state and after being heated at various temperatures and periods. The modes of fixation were also in every case 1 hour in the water bath and 4 hours in the icebox.

These specific complement-fixing antibodies were found to be, without exception, persistently thermostable. In order to raise the coagulation point, the serums subjected to a temperature of 65 C. and higher were previously diluted 1:10 with salt solution.

[5] For preliminary report see Kahn, R. L.: Proceed. Soc. for Exp. Biol. and Med., 1921, 18, p. 4.

[6] Kahn, R. L.: Jour. Exper. Med., 1921, 34, p. 217.

Table 4 indicates that these antibodies are capable of withstanding a temperature of 70 C. for 15 minutes. The results of heating periods of 1 hour at 70 C. and a half hour at 75 C. have not been constant and for this reason are not recorded. At times there was practically no antibody destruction at these temperatures and periods and at other times there was about 25% or more antibody destruction. It would appear that the thermal destructive temperature for these antibodies lies between 70 C. and 80 C.

TABLE 3

THE THERMOSTABILITY OF COMPLEMENT-FIXING ANTIBODIES PRODUCED BY PROTEIN IMMUNIZATION

Time and Temperature of Heating	Mode of Fixation	Serum of Rabbit Immunized with Edestin, C c									Total Plus Signs
		0.01	0.007	0.004	0.003	0.002	0.001	0.0005	0.0003	0.0001	
0	Water bath*....	2	2	1	1	—	—	—	—	—	6
	Icebox.....	3	3	1	1	—	—	—	—	—	8
5 min. at 56 C.	Water bath.....	3	3	1	1	1	—	—	—	—	9
	Icebox.....	4	4	2	2	—	—	—	—	—	12
15 min. at 56 C.	Water bath.....	3	2	1	1	1	—	—	—	—	8
	Icebox.....	4	4	2	2	—	—	—	—	—	12
30 min. at 56 C.	Water bath.....	3	3	1	1	—	—	—	—	—	8
	Icebox.....	4	4	3	2	—	—	—	—	—	13
60 min. at 56 C.	Water bath.....	3	3	1	1	—	—	—	—	—	8
	Icebox.....	4	4	3	2	—	—	—	—	—	13
15 min. at 62 C.	Water bath.....	3	3	1	1	—	—	—	—	—	8
	Icebox.....	4	4	3	2	—	—	—	—	—	13
30 min. at 62 C.	Water bath.....	3	3	1	1	—	—	—	—	—	8
	Icebox.....	4	4	3	2	—	—	—	—	—	13
60 min. at 62 C.	Water bath.....	3	3	1	1	—	—	—	—	—	8
	Icebox.....	4	4	3	2	—	—	—	—	—	13
30 min. at 65 C.	Water bath.....	3	3	1	1	—	—	—	—	—	8
	Icebox.....	4	4	3	2	—	—	—	—	—	13
60 min. at 65 C.	Water bath.....	3	3	1	1	—	—	—	—	—	8
	Icebox.....	4	4	3	2	—	—	—	—	—	13
120 min. at 65 C.	Water bath.....	3	3	1	1	—	—	—	—	—	8
	Icebox.....	4	4	1	1	—	—	—	—	—	10

* The period of water bath fixation was 1 hour; of icebox fixation, 4 hours.

Summary of Results Obtained With Protein Immune Serums.—A study of the velocity of destruction of complement-fixing antibodies present in the serums of rabbits immunized with purified proteins showed these antibodies to be highly thermostable. Heating for 2 hours at 65 C. or for 15 minutes at 70 C. produced little effect on these antibodies. Prolonged heating at 70 C. and 75 C. resulted in varying degrees of antibody destruction. The tendency for stronger

binding of complement after a fixation period of 4 hours in the icebox compared with one hour in the water bath was noted also with these specific antibodies.

THE EFFECT OF HEAT ON COMPLEMENT-FIXING ANTIBODIES PRODUCED BY BACTERIAL IMMUNIZATION

The marked difference in the behavior toward heat of syphilitic complement-fixing substances compared with specific antibodies obtained after protein injections raised the question of the behavior toward this agent of similar antibodies obtained after bacterial

TABLE 4

THE THERMAL DESTRUCTION OF SPECIFIC COMPLEMENT-FIXING ANTIBODIES

Immune Serum Rabbit	Protein Used in Immunization	Time and Temperature of Heating	Serum, C c									Total Plus Signs
			0.01	0.007	0.004	0.003	0.002	0.001	0.0005	0.0003	0.0001	
1	Edestin	30 minutes at 56 C. ...	4	4	4	4	4	2	1	—	—	23
		15 minutes at 70 C. ...	4	4	4	4	4	1	1	—	—	22
		15 minutes at 80 C. ...	—	—	—	—	—	—	—	—	—	0
4	Edestin	30 minutes at 56 C. ...	3	2	1	1	—	—	—	—	—	7
		15 minutes at 70 C. ...	3	2	1	1	—	—	—	—	—	7
		15 minutes at 80 C. ...	—	—	—	—	—	—	—	—	—	0
A	Phaseolin	30 minutes at 56 C. ...	4	3	2	—	—	—	—	—	—	9
		15 minutes at 70 C. ...	4	2	1	—	—	—	—	—	—	7
		15 minutes at 80 C. ...	—	—	—	—	—	—	—	—	—	0
B	Phaseolin	30 minutes at 56 C. ...	4	4	4	4	4	4	1	—	—	25
		15 minutes at 70 C. ...	4	4	4	4	4	4	1	—	—	25
		15 minutes at 80 C. ...	—	—	—	—	—	—	—	—	—	0

Method of fixation, 4 hours at icebox temperature.

immunization. It was furthermore desired to determine to what extent the mode of fixation affected the velocity of thermal destruction of these antibodies.

The bacterial antigens employed were B. typhosus, B. mallei and B. abortus. The typhoid culture was obtained from the bacteriologic division of these laboratories and the antigen was prepared from 24 hour agar slants. The bacterial suspension was heated for 1 hour at

56 C. and 0.5% phenol added as a preservative. Cultures of B. mallei and B. abortus were kindly furnished us by Mr. I. F. Huddleson of the Bacteriological Department of the Michigan Agricultural College. Antigen suspensions of these organisms were prepared as in the case of B. typhosus. After regular antigenic titrations in the presence of their specific serums, these antigens were finally employed in such dilutions that 0.1 c c—the quantity used in the tests—contained 3 antigenic units and 0.3 c c were neither anticomplementary nor hemolytic.

TABLE 5

THE EFFECT OF THE MODE OF FIXATION ON THE VELOCITY OF THERMAL DESTRUCTION OF COMPLEMENT-FIXING ANTIBODIES PRODUCED BY IMMUNIZATION WITH B. ABORTUS

Time and Temperature of Heating	Temperature of Fixation (Period = 1 Hr.)	Immune Abortion Serum (Bovine), C c									Total Plus Signs
		0.01	0.007	0.004	0.003	0.002	0.001	0.0005	0.0003	0.0001	
0	Water bath.....	4	4	4	4	4	—	—	—	—	20
	Icebox.....	4	4	4	4	4	—	—	—	—	20
30 min. at 56 C.	Water bath.....	4	4	4	4	3	—	—	—	—	19
	Icebox.....	4	4	4	4	4	—	—	—	—	20
60 min. at 56 C.	Water bath.....	4	4	4	4	3	—	—	—	—	19
	Icebox.....	4	4	4	4	4	—	—	—	—	20
15 min. at 65 C.	Water bath.....	4	4	4	4	3	—	—	—	—	19
	Icebox.....	4	4	4	4	4	—	—	—	—	20
15 Min. at 70 C.	Water bath.....	4	4	4	3	2	—	—	—	—	17
	Icebox.....	4	4	4	3	2	—	—	—	—	17
15 min. at 75 C.	Water bath.....	2	1	1	1	1	—	—	—	—	6
	Icebox.....	2	1	1	1	1	—	—	—	—	6

With regard to the antiserums, in the case of B. typhosus and B. mallei, rabbit serums were employed; in the case of B. abortus, bovine serum.

The complement-fixation tests were carried out in practically every detail as with the syphilitic and protein immune serums, except that the methods of fixation were 1 hour in the water bath and 1 hour (instead of 4 hours) in the icebox. Studies on the rate of fixation of complement with bacterial antiserums are at present being carried out in this laboratory, and it was deemed best to employ a uniform period of 1 hour both at water bath and icebox temperatures.

Table 5 records the velocity of destruction of complement-fixing antibodies (bovine) obtained after immunization with B. abortus. It appears from this table that these antibodies begin to break down when subjected to a temperature of 70 C. The antityphoid and anti-glanders serums gave similar results. These experiments also indicated

that the phenomenon of fixation goes on equally well at water bath and icebox temperatures.

Table 6 gives the results of an experiment carried out with the three bacterial antiserums. No antibody destruction was noted after heating these serums for 1 hour at 65 C. Heating for 1 hour at 70 C. showed variable degrees of destruction, and one-half hour at 75 C. was highly destructive to these antibodies.

TABLE 6

THE THERMAL DESTRUCTION OF COMPLEMENT-FIXING ANTIBODIES PRODUCED BY BACTERIAL IMMUNIZATION

Immune Serum	Organism Used in Immunization	Time and Temepra-ture of Heating	Serum, C c									Total Plus Signs
			0.01	0.007	0.004	0.003	0.002	0.001	0.0005	0.0003	0.0001	
Rabbit	B. typhosus	0.....	4	4	4	4	3	1	1	—	—	21
		60 minutes at 65 C. ...	4	4	4	4	3	1	1	—	—	21
		60 minutes at 70 C. ...	4	4	4	2	1	1	1	—	—	17
		30 minutes at 75 C. ...	4	3	2	1	1	1	—	—	—	12
		60 minutes at 75 C. ...	4	3	2	1	1	—	—	—	—	11
Rabbit	B. mallei	0.....	4	4	4	3	2	1	1	1	—	20
		60 minutes at 65 C. ...	4	4	4	3	2	1	1	1	—	20
		60 minutes at 70 C. ...	4	2	1	1	1	—	—	—	—	9
		30 minutes at 75 C. ...	2	1	1	1	—	—	—	—	—	5
		60 minutes at 75 C. ...	1	1	—	—	—	—	—	—	—	2
Bovine	B. abortus	0.....	4	4	4	4	4	4	4	2	2	32
		60 minutes at 65 C. ...	4	4	4	4	4	4	4	2	1	31
		60 minutes at 70 C. ...	4	4	4	4	4	4	2	1	1	28·
		30 minutes at 75 C. ...	4	2	1	1	..	..	..	..	..	8
		60 minutes at 75 C. ...	2	1	..	..	..	..	..	..	..	3

Method of fixation, 1 hour at icebox temperature.

Summary of Results Obtained With Bacterial Immune Serums.—The velocity of destruction by heat of bacterial complement-fixing antibodies was investigated. The following antiserums were employed: antityphoid (rabbit), antimallei (rabbit), and antiabortion (bovine). The results of complement-fixation studies conducted with raw serums and the same serums heated at different temperatures and periods indicated that these antibodies are quite as thermostable as those obtained on protein immunization. With regard to the mode of fixation,

the findings indicate that the difference between 1 hour fixation in the icebox from the same period in the water bath is not marked.

DISCUSSION

The observation that specific complement-fixing substances are capable of withstanding a temperature of 65 C. while so-called syphilitic antibodies are incapable of withstanding much lower temperatures is, in our opinion, significant. It places specific complement-fixing antibodies in a class with specific agglutinins and precipitins, and syphilitic complement-fixing substances apparently in a class by themselves. And yet, we question whether our data indicates that the complement-fixing antibodies in syphilis are essentially different from those found in other immune serums. Of the types of immune serums investigated, only those which were tested with nonspecific antigens showed the presence of thermolabile antibodies. Is it not likely, therefore, that the apparent thermolability of these antibodies is due to the nature of the antigens? This view is strengthened by the fact that both the mode of fixation and the type of antigen employed, markedly affect the complement binding power of heated syphilitic serums. In our opinion, in order to prove with reasonable certainty whether or not a given antibody is thermostable, the immune serum should be tested with a freshly prepared specific antigen.

On the other hand, this difference in the behavior toward heat by the two types of antibodies suggests that the phenomenon of complement fixation in syphilis is more or less distinct from complement fixation with specific antigens. And it is not unlikely that the negative complement-fixation findings in bacterial infections, such as tuberculosis and gonorrhea, in which a Wassermann procedure was employed, are inconclusive. For, the applicability of this procedure in syphilis does not necessarily imply its successful general application in other infectious diseases.

SUMMARY

It was shown that thermal destruction of syphilitic complement-fixing substances is markedly influenced by the mode of fixation. When fixation was carried out for 1 hour at water bath temperature, the heating of serum for 30 minutes at 56 C. showed in a total of 87 serums tested an average antibody destruction of 32%. When fixation was carried out for 4 hours at icebox temperature, some serums showed a slight loss, others, no loss and still others, a considerable gain in

antibody content, with the result that the average finding of the 87 serums tested represented a gain of 10%. Heating syphilitic serums for 20 minutes at 62 C. gave an average of 75% antibody destruction, with water bath fixation, and 46%, with icebox fixation.

The type of antigen employed was also found to influence thermal destruction of these antibodies. Heating serums for 30 minutes at 56 C. showed either little antibody destruction or an apparent gain in antibody content with 2 alcoholic extract antigens and one cholesterinized antigen and icebox fixation, while even with this mode of fixation, considerable destruction was noted at this temperature and period when employing the Noguchi antigen (table 2).

Finally, it was shown that complement-fixing antibodies obtained on protein or bacterial injections were comparatively thermostable. These antibodies were found to be capable of withstanding a temperature of 65 C. Temperatures of 70 C. and 75 C. showed varying degrees of antibody destruction—somewhat more so in the case of bacterial immune bodies than those obtained on protein injections.

With regard to the effect of the mode of fixation on specific antibody destruction due to heating, no marked difference was observed between water bath and icebox temperatures.